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Lipid Oxidation and Pigment Changes in Raw Beef

SUMMARY—Two major sources of nonmicrobial deterioration in prepackaged raw meats are the development of off-odors and off-colors. The relationship of these changes to polyunsaturated fatty acid oxidation in the meat was investigated. Lipid oxidation was measured by the thiobarbituric acid test; pigment changes, by reflectance spectrophotometry. Lipid oxidation was found to produce detectable off-odors in raw and subsequently cooked beef. Anaerobic packaging to prevent oxidation of myoglobin and in turn, lipids, appeared to be useful only if packaging (and oxygen removal) could be carried out rapidly and if meat contained sufficient enzyme activity to establish anaerobic conditions quickly and to completely reduce metmyoglobin. Propyl gallate and butylated hydroxyanisole, even under aerobic conditions, offered substantial protection to the fresh meat pigments and at the same time effectively inhibited lipid oxidation.

INTRODUCTION

A COMMON PROBLEM in marketing prepackaged meats is the development of an undesirable brown color after the meat has been cut. This is due to oxidation of the red meat pigments oxymyoglobin (MbO_2) and myoglobin (Mb) to the brown ferric metmyoglobin (MetMb). In addition, there is evidence (Younathan et al., 1960; Brown, et al., 1963) that ferric heme pigments can catalyze oxidation of the tissue lipids in meat. Free radical intermediates from this reaction can decompose hemes, using loss of color (Haurowitz et al., 1941). In cooked meats, lipid oxidation produces a stale or "rancid" odor. Rancid odors associated with lipid oxidation would also be expected in raw meat, but this problem has not yet been investigated.

To retard both pigment and lipid oxidation in raw meat, two approaches may be taken: (1) preventing MetMb formation to eliminate the lipid oxidation catalyst, and (2) preventing lipid oxidation, by means of an antioxidant to protect the pigment from lipid oxidation intermediates.

The first approach focuses attention on enzymatic reduction of MetMb. Stewart et al. (1965a) have shown that enzymes in raw meat can reduce MetMb under anaerobic conditions. Wrapping the meat in an oxygen-impermeable film usually produces anaerobiosis and pigment reduction. When MetMb has been completely reduced, provided no additional oxygen enters the package, no further oxidation of pigment or lipids should occur. One disadvantage of this approach is that reduced Mb is purple, which is not the typical color the consumer associates with fresh meat. At the present time most meat is packaged in air permeable films. The pigment is in the familiar bright red form of MbO_2. The free exchange of oxygen, however, makes the meat more susceptible to oxidation of both pigment and lipids. An antioxidant might inhibit lipid oxidation and still permit aerobic packaging. If this type of treatment also afforded pigment protection, it might be more advantageous from the standpoint of consumer appeal.

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The present work was done to determine whether lipid oxidation produces rancid odors in raw beef and to determine whether an antioxidant and/or anaerobic packaging can prevent both lipid and pigment oxidation.

EXPERIMENTAL PROCEDURE

BEEF TOP ROUND was used, purchased locally with no knowledge of its previous history. The meat was trimmed of adipose tissue and ground twice in a Hobart grinder fitted with stainless steel and chrome plated parts to avoid contamination with metal ions. Thirty ppm chlortetracycline was thoroughly mixed with the meat immediately after grinding. This eliminated the possibility of off-odors due to bacterial spoilage. The antioxidants tested were butylated hydroxyanisol (BHA), 0.01%; propyl gallate (PG), 0.01%; sodium tripolyphosphate (PP), 0.5%. PG and PP were added as aqueous solutions; BHA as an emulsion (Sustane E, Universal Oil Products Co.). Samples of uniform weight and surface area were packaged either in impermeable Saran (Dow,) or in "Prime Wrap" (Goodyear), which is a highly oxygen-permeable film commonly used by supermarkets for wrapping meat cuts. They were stored at approximately 4°C on a tray in a single layer.

MetMb was measured by reflectance spectrophotometry as described by Stewart et al. (1965b) on a Bausch and Lomb "Spectronic 505" recording spectrophotometer. Lipid oxidation was measured by the thiobarbituric acid (TBA) test (Tarladgis et al., 1960). Results are expressed as "TBA number," meaning mg of malonaldehyde per 1,000 g of meat. A trained panel evaluated the samples for rancid odors. Significance of differences was tested by the Wilcoxon matched-pairs signed-ranks test (Siegel, 1956).

RESULTS

Effect of lipid oxidation on odor of meat

The first experiment was designed to determine whether lipid oxidation actually produces off-odors in raw meat. Samples were stored in the oxygen-permeable wrap. Table 1 gives an example of the results. The BHA- and PG-treated samples were rated significantly higher on sensory scores than the control samples. A TBA number of 0.5 to 1.0 in cooked meat is considered the range in which odor can first be detected (Tarladgis et al., 1959). Odor was first evaluated in raw meat and later in samples that had been cooked after several days of storage in the raw state. Cooking did not destroy the rancid odor. To determine whether BHA and PG could continue protection after the meat was cooked, samples were stored for two days after cooking and assayed again. The results, not presented here, showed that cooking did not destroy the antioxidant properties of these compounds.

Table 1—Effect of antioxidants on odor scores and TBA numbers of raw and subsequently cooked beef.

Experiment	Sample	Days stored	Odor score[1]	TBA No.
1	Control (raw)	2	3.5	5.7
1	Polyphosphate (raw)	2	3.9	4.8
1	BHA (raw)	2	4.9	0.6
2	Control (raw)	8	3.5	12.5
2	PG (raw)	8	5.6	0.9
2	Control (cooked)[2]	8	3.7	16.4
2	PG (cooked)[2]	8	5.4	0.5

[1] All samples with BHA or PG significantly different from controls. Sample with BHA significantly different from sample with polyphosphate. A score of 6 = no detectable off odor; 1 = strong off odor.
[2] Cooked after 8 days of storage raw.

Sodium tripolyphosphate has been used effectively as an antioxidant in cooked meat and fish (Tims et al., 1958; Ramsey et al., 1963). Johnson (1966) found that in raw meat, the phosphate groups were hydrolyzed presumably by phosphatases in the muscle. In order to use PP as an antioxidant it was necessary to first heat the meat to at least 70°C. The data in Table 1 further illustrate its ineffectiveness as an antioxidant in raw meat.

Effect of antioxidants and wrapping material on lipid oxidation and MetMb formation

Table 2 shows the effects of antioxidants on lipid oxidation (TBA number) and MetMb formation in top round samples wrapped in both oxygen permeable and oxygen impermeable films. In this experiment Saran wrap was sufficient to reduce MetMb and prevent its accumulation. By the second day the meat in these packages had turned a deep purple color. The TBA numbers for the untreated sample, while high for essentially anaerobic storage, did not change to any extent after the first day. Once the pigment was reduced and little residue oxygen remained in the package, no further lipid oxidation took place. The high TBA number probably developed while the meat was being packaged and before the pigment had reduced.

TBA numbers were quite low in the sample with added BHA. It was added shortly after the meat was ground so that time involved in preparation and for MetMb reduction would not affect lipid oxidation in this sample. A later experiment helped to substantiate this. TBA tests were run immediately after grinding the meat and then again shortly after packaging. During this time the TBA number of unprotected samples had more than quadrupled. Keskinel et al. (1964) found a similar increase in TBA number immediately after grinding raw beef.

Wrapping the meat in Saran did not always result in MetMb reduction and lipid oxidation inhibition. When samples that did not undergo reduction after packaging in Saran were tested for MetMb-reducing activity (MRA) by the method of Stewart et al. (1965b), activity was barely detectable. Anaerobic packaging can apparently be useful only if sufficient reducing activity is present in the meat.

In examining the effects of antioxidants on lipid and pigment oxidation, the ineffectiveness of PP is again illustrated (Table 2). However, in all cases both BHA and PG effectively protected against lipid oxidation. With aerobic packaging they also offered substantial protection against MetMb formation. After one week, samples containing either of these antioxidants and packaged in Prime Wrap were still a good red color.

Table 2—Effect of antioxidants and wrapping material on MetMb development and lipid oxidation in refrigerated raw beef.

Experiment	Antioxidant	Wrapping material	Days stored	TBA No.	% MetMb
1	None	Saran	1	2.6	52
	None	Saran	7	3.0	0
	None	Prime Wrap	1	5.0	49
	None	Prime Wrap	7	13.3	66
	Polyphosphate	Saran	1	2.1	29
	Polyphosphate	Saran	7	1.9	0
	Polyphosphate	Prime Wrap	1	3.5	40
	Polyphosphate	Prime Wrap	7	7.7	50
	BHA	Saran	1	0.6	48
	BHA	Saran	7	0.5	0
	BHA	Prime Wram	1	0.7	31
	BHA	Prime Wrap	7	0.6	33
2	None	Prime Wrap	8	6.2	52
	BHA	Prime Wrap	8	0.4	36
	PG	Prime Wrap	8	0.7	36

DISCUSSION

THESE RESULTS have demonstrated the importance of lipid oxidation in raw beef in relation to both color and odor. The odor produced from this reaction in raw beef had not previously been recognized as resulting from lipid oxidation. The fact that the odor remains after cooking the meat places further importance on this type of spoilage.

Anaerobic packaging can effectively prevent MetMb formation and rancidity, providing sufficient MRA is present in the meat. The great variability in MRA between different samples of meat suggests a need for research into factors limiting this enzyme action. Genetic or environmental factors or slaughter conditions undoubtedly play a part in this variability. Work is presently in progress to determine the substrate(s) for MetMb-reducing enzymes in meat. Addition of suitable substrates either directly to the meat or indirectly to the whole animal could result in meat with uniformly high MRA.

Anaerobic packaging is unnecessary if an antioxidant is added to the meat. BHA and PG inhibit lipid oxidation and retard MetMb formation. In this way, the meat pigment can be in the familiar oxygenated form. This method offers the additional advantage of preventing rancidity in the cooked product. A number of recent studies (among them, Lewis et al., 1962, and Roubal et al., 1966) have shown that intermediates from oxidizing lipids can damage proteins and enzymes. Antioxidants may exert their effect on meat color by protecting reducing enzymes as well as heme pigments from damage due to lipid oxidation intermediates.

We performed additional experiments to investigate this possibility, using the method of Stewart et al. (1965a). While more MRA was retained in stored samples treated with an antioxidant, the effect was not great enough to completely account for the amount of MetMb inhibition that occurred. In addition to protecting enzyme proteins, antioxidants may also protect heme proteins. Heme pigments are more susceptible to oxidation if the globin has been denatured (Lemberg et al., 1949). Studies by Nishida et al. (1965) and Little et al. (1968) indicated, however, that destruction of the heme moiety of hemoglobin may be the main point of attack by lipid hydroperoxides rather than denaturation of the protein portion. Measurement of total pigment might provide evidence of heme protection.

Initial MRA may be important to antioxidant treated samples, too. During these experiments enzymically "active" samples were observed to brown more readily in aerobic packaging than less active samples. At the same time, antioxidant-treated "active" samples remained a brighter red than the same samples from a less active piece of meat. Antioxidants may prevent oxidation of the heme iron, although this is more difficult to substantiate.

Samples of meat with a very high pH (6.2) have been found to remain red under aerobic conditions even without an antioxidant. Rancidity does not develop in this case. Such meat is usually associated with extremely high reducing activity. It is apparently just on the border line of "dark-cutting" beef, but it does oxygenate and it is not unduly sticky in texture. Finding a means of producing animals to give this type of meat would be extremely valuable. Perhaps some of the pre- and post-slaughter treatments proposed for reducing PSE in pork would be helpful with beef also.

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